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Starter N and P Fertilizers Have Dissimilar Effects on Native Mycorrhizal and *Bradyrhizobial* Symbiosis of Four Promiscuous Soybean Varieties in Acid Soils of Cameroon

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Summary

*Influence of starter nitrogen (N) and phosphorus (P) applications on mycorrhizal colonization and nodulation of soybean (*Glycine max *(L.) Merill) was investigated at two sites of humid forest margins of Cameroon. Four soybean varieties, TGX 1838-5E (var1), TGX 1879-7E (var2), TGX 1828-4E (var5) and TGX 1805-13F (var6), were grown for four months in fields from different fallow ages, in a factorial combination of 30 kg N.ha^{-1} and 30 kg P.ha^{-1}, with five replicates, in a randomized complete block design. Soil acidity varied strongly with site, being 5.9 and 4.5, at Nkometou and Mengomo, respectively. Fractional mycorrhizal colonization (FMC) was not affected by soil pH. FMC significantly varied among fields between 20% and 40%, was significantly reduced by P fertilization while effect of N amendment was contrasted. Nodulation was strongly influenced by soil pH: high nodulation in Nkometou but extremely low in Mengomo. In Nkometou, early maturing soybean varieties (var2 and var5) yielded higher nodule number and mass than late-maturing ones (var1 and var6). Var2 exported the highest biological N and var6 the lowest. Plant P uptake only differed among fields. Nodulation and grain yield did not respond to the fertilizer rates. Negative, highly significant correlations were established between nodule number and mass (r= - 0.726; p< 0.0001; n= 68), between nodule number and FMC (r= - 0.682; p< 0.0001; n= 68). However, a positive and highly significant correlation was obtained between FMC and nodule mass (r= 0.976; p< 0.0001; n= 68). Such biological reactions to fertilization could be attributed to effective indigenous arbuscular mycorrhizal fungi and* bradyrhizobia.

Résumé

Les engrais azoté et phosphaté de démarrage ont des effets dissimilaires sur les champignons mycorhiziens et les souches de *Bradyrhizobium* indigènes sur sols acides du Cameroun

*L'influence d'applications d'engrais azoté et phosphaté de démarrage sur la colonisation mycorhizienne et la nodulation de soja (*Glycine max *(L.) Merill) a été investiguée dans deux sites des marges de forêt humide. Quatre variétés de soja, TGX 1838-5E (var1), TGX 1879-7E (var2), TGX 1828-4E (var5) and TGX 1805-13F (var6), ont été cultivées pendant quatre mois dans les champs d'âges différents, dans une combinaison factorielle de 30 kg N.ha^{-1} et 30 kg P.ha^{-1}, avec cinq répétitions, dans un dispositif en bloc complètement randomisé. L'acidité des sols diffère fortement suivant le site, soit respectivement 5,9 et 4,5 à Nkometou et Mengomo. Le taux de colonisation mycorhizienne (TCM) n'a pas été affecté par le pH. Le TCM a significativement varié entre les champs entre 20 et 40%, a significativement été réduit par la fertilisation phosphatée alors que l'effet de l'amendement azoté a été contrasté. La nodulation a été fortement influencée par le pH: forte nodulation à Nkometou et extrêmement faible nodulation à Mengomo. A Nkometou, les variétés précoces de soja (var2 et var5) ont produit un plus grand nombre et une forte masse de nodules que les variétés tardives (var1 et var6). La variété précoce Var2 a exporté le plus d'azote biologique et la Var6 le moins. L'absorption de P par la plante a différé seulement entre les champs. La nodulation et le rendement en grain n'ont pas varié suivant le taux de fertilisation. Des corrélations fortement significativement et négatives ont été établies entre le nombre et la masse de nodules (r= - 0.726; p< 0.0001; n= 68), entre le nombre de nodules et le TCM (r= -*

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0.682; p< 0.0001; n= 68). Cependant, une corrélation fortement significative et positive a été obtenue entre le TCM et la masse de nodules (r= 0.976; p< 0.0001; n= 68). De telles réactions à la fertilisation pourraient attribuées aux symbionts indigènes de champignons mycorhiziens arbusculaires et de Bradyrhizobium*.*

Introduction

Annually, grain legumes cultivated worldwide on about 1.5 million km^2 of land per year (9) fix about 44 – 66 million tons of biological nitrogen in symbiosis with *rhizobia* and *bradyrhizobia*, providing almost half of all nitrogen used in agriculture (2). Soybean (*Glycine max* (L.) Merill), the largest oilseed crop worldwide accounting for more than 50% of the world oilseeds production, is one of the most important legume plants owing to its high protein and oil contents for human, poultry and swine consumption, sometimes up to 40% (22). It has also been used as a source for bio-diesel fuels (13). Worldwide soybean production is estimated at 211 million metric ton per year with only 2% from Africa.

Attempts to introduce improved soybean varieties in humid forests of West and Central Africa have been tempered by low yields (26). Due to the inherently low fertile acid soils, soybean plants often show nitrogen (N) and phosphate (P) deficiency symptoms during early growth stages, thereby, suggesting that starter N and/or P fertilization may improve soybean yields in humid forests (4). However, applications of N and P fertilizers to improve soybean productivity have resulted in varying and contradictory results (14, 27).

Soybean varieties are symbiotically promiscuous with native arbuscular mycorrhizal fungi (AMF) and indigenous soil bradyrhizobia (1). Association with *Bradyrhizobia* provides biologically converted atmospheric nitrogen in nodules, while AMF, by improving soybean scavenging roots' ability, supplies the much needed and scarce P for energy required for biological N_2 fixation. Consequently, mycorrhizal and effectively nodulating soybean plants could better establish on acid poor-nutrient soils that prevail in the tropics without fertilization.

However, chemical soil conditions may have various effects on symbiotic activities. In general, low pH reduces nodulation while having a fluctuating effect

Figure 1: Localization of IITA benchmark and both experimental sites.

on mycorhization (17). High N levels (> 1mM) also drastically decrease nodulation and nitrogenase activity (16). Many nitrogenous fertilizers have also been reported to reduce mycorrhizal colonization (10, 32). Interactions between N and P fertilizers also exist with a more marked effect of P in N-sufficient plants than in N-deficient ones (6). Hampered mycorrhizal colonization by high P fertilization may vary with host species, mycorrhizal fungal strains and environmental factors (25). Following these inconsistent results, therefore, the introduction of exotic soybean varieties in humid forests requires an initial assessment of the effects of fertilization on symbiotic activities of native symbionts. Thus, the productivity of introduced promiscuous soybean varieties could depend both on residual soil N and P contents and on persistence of efficient symbionts in the humid forest sites. The objective of this study was to assess the effect of starter N and P fertilizers on bradyrhizobial and mycorrhizal fungal symbiotic activities and productivity of four promiscuous varieties of *Glycine max* grown in two soils with contrasted acidity in the Center region of Cameroon (Figure 1).

Material and methods

Study areas

The study was carried out at two benchmark sites of the International Institute of Tropical Agriculture (IITA), Nkometou and Mengomo. Elevation, rainfall, soil types and chemical characteristics are shown in table 1. Nkometou (3°52'N; 11°26'E), located 35 km north of Yaounde (3°42'N;12°67'E), is a densely populated rural area of high agricultural intensity; fallowing dominated by the exotic weed *Chromolaena odorata* (L.) King and Robinson (Asteraceae) has been greatly shortened. In contrast, Mengomo (2°35'N; 11°03'E), situated 165 km south of Ebolowa (2°56'N; 11°21'E) and 305 km from Yaounde, is a sparsely populated rural area with still large tracts of intact evergreen forest. The climate is equatorial of the Guinean type with two distinct wet seasons, culminating in May and October.

Plant materials

Four varieties of soybean provided by IITA Humid forest station, Mbalmayo, Cameroon, were used, viz. TGX 1838-5E (var1), TGX 1879-7E (var2), TGX 1828-4E (var5) and TGX 1805-13F (var6). Var2 and var5 were early maturing; var1 and var6 were late maturing.

Experimental design

At each site, differently aged fallows of *C. odorata* (1 to 3 years) were randomly selected. In Mengomo, it fluctuates from 3 to 6 years, in addition to 16 years old young secondary forests. Selected experimental field plots were 16 m by 28 m large and divided into 8 m by 3.5 m plots and set up in a 4 x 2 x 2 factorial combination in a RCMD with five replicates.

Planting and harvesting

Seeds (250,000 plants. ha^{-1} density) were sown at the onset of the small rainy season (mid-march 2008), simultaneously with surface application of triple superphosphate, in 4 cm depth at 4 cm bands, away from the planting line. Two weeks later, urea was similarly surface applied. Manual weeding was performed 30 and 59 days after sowing before flowering and harvesting four months later. Soybean plants with intact nodules were harvested per plot (2.5 m by 7 m) cleaned root systems with nodules and whole plants oven-dried at 70 °C for a week before separated weighing.

Root staining and assessment of mycorrhizal colonization

Five mature soybean plants with profuse whitish roots were randomly uprooted from the harvesting area. About 2.5 g portions of fine roots were collected per plant. 0.5 g portions of root samples were stained with fuchsin acid in lactic acid and destained (25), followed by microscopic assessment. Fractional mycorrhizal colonization (FMC) was assessed by the gridline intersect method under a dissecting microscope at 40x (11).

Soil sampling and plant analyses

Before applying fertilizers to the experimental plots, ten core soil samples (0 – 20 cm) were collected for chemical analysis. N and P were assessed after ash-drying followed by colorimetric techniques (3).

Statistical analyses

Data were analyzed in SAS release version 6 (29) using the General linear model procedure after separation of both sites, checking for normal distribution followed by arc sin square transformation for FMC, square root transformation for nodule number and mass were and log transformation for grain yield. Data of nodule number and mass of Mengomo had too many zeroes and were analyzed by the Kruskall-Wallis non parametric test. Means of dependent variables were

Table 1
Elevation, rainfall, soil types and chemical characteristics of soils (0 – 20 cm) in studied sites

Localities	Nkometou	Mengomo
Elevation (m.a.s.l)	596	620
Rainfall (m)	1643	1820
Clay types	very fine clayey	clayey
Soil types	Kandiudult	Kandiudox
pH (water)	5.98	4.54
Carbon (%)	1.50	1.10
Nitrogen (%)	0.127	0.0914
C/N	11.8	12.0
Available P (ppm)	8.14	6.77
Ca (cmol.kg^{-1})	2.69	0.680
Mg (cmol.kg^{-1})	0.712	0.238
K (cmol.kg^{-1})	0.084	0.102
Al (cmol.kg^{-1})	0.044	1.24

Note: Available P by the Mellich-III method.

Table 2
General linear model of arc sin square root transformed fractional mycorrhizal colonization (FMC) of soybean roots, square root nodule number (Nn) and square root mass (Nm), log grain yield (kg/ha), shoot N and P uptake (mg/plant) in Nkometou as influenced by fields, variety and fertilization (Probabilities values only)

Sources	FMC	Nn	Nm	Grain yield	Plant N	Plant P
Field	**0.002****	0.918ns	0.289ns	0.121ns	0.0839ns	**0.0026****
Variety	0.124ns	**0.030***	**0.017***	0.757ns	**0.0047****	0.254ns
Fertilization	**0.027***	0.643ns	0.565ns	0.664ns	0.787ns	0.856ns
Field by variety	0.521ns	0.610ns	0.560ns	0.939ns	0.166ns	0.968ns
Field by fertilization	0.829ns	0.869ns	0.961ns	0.401ns	0.492ns	0.967ns
Variety by fertilization	0.829ns	0.995ns	0.950ns	0.999ns	0.479ns	0.993ns
Field by variety by fertilization	0.819ns	0.994ns	0.982ns	0.714ns	0.885ns	0.998ns

separated by Duncan's multiple range tests and Pearson's rank correlation coefficients calculated between them.

Results

Both sites considerably varied in soil pH and nutrient status. Mengomo' soils were strongly acidic, lower in N, Ca and had higher exchangeable Al than Nkometou' soils which were nearly neutral. Available P was equally very low at both sites (Table 1).

All soybean roots were mycorrhizal by native AM fungi, in the range 20 – 40%. They were colonized by oval to rectangular vesicles with straight or tortuous internal hyphae; non-septate external hyphae were also abundant without auxiliary bodies. Arbuscules and hyphal coils were not observed.

Fractional mycorrhizal colonization of soybean roots highly significantly varied with field ($p < 0.001$) and was significantly affected by fertilization ($p < 0.05$) in both sites but neither by variety nor by most interactions (Table 2). FMC significantly increased with increasing fallow age and in forest regrowth (Figure 2).

Fractional mycorrhizal colonization was significantly reduced by P fertilization at both sites, in the range 11 – 15%. Following N fertilization, FMC did not change in Nkometou but decreased in Mengomo. NP fertilization did not significantly affect FMC in Nkometou but reduced it in Mengomo (Figure 3).

Nodulation of all four varieties markedly varied with

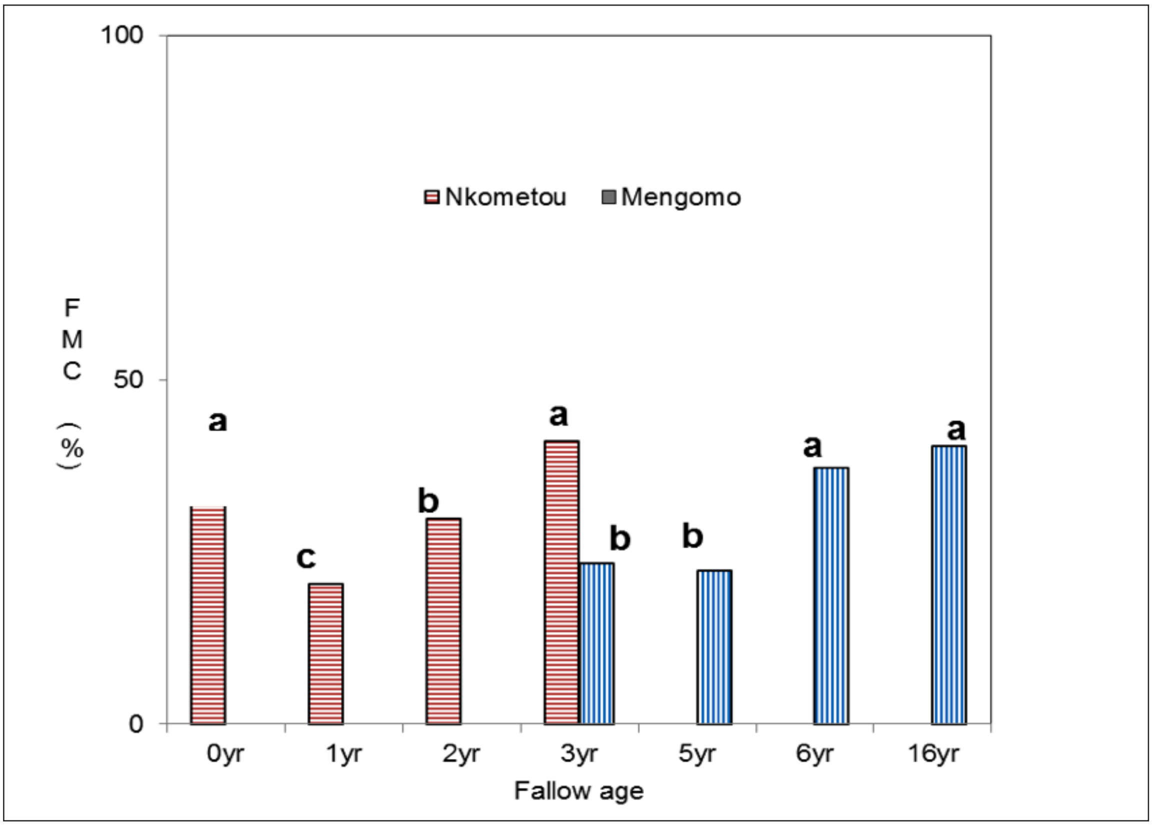

Figure 2: Fractional mycorrhizal colonization (FMC) of soybean roots with fields from fallow of different ages in Nkometou (open bars) and Mengomo (Shaded bars). Bar graphs followed by the same letter are not significantly different at 5% level of significance.

Table 3
Change in nodule number (# per plant), nodule mass and N uptake (mg per plant) among varieties in Nkometou and Mengomo

Varieties	Nkometou Nodule			Mengomo Nodule
	Number	Mass	N uptake	Number
Var1 (TGX 1838-5E)	17.4a	155.7bc	3.57bc	0.691b
Var2 (TGX 1879-7E)	16.3a	265.7a	4.03a	0.0154c
Var5 (TGX 1828-4E)	15.7a	204.5ab	3.9ab	1.50a
Var6 (TGX 1805-13F)	4.41b	75.2c	3.28c	0.0222c

*Figures in columns followed by the same letter are not significantly different at 5% level of significance.

site: High nodulation in Nkometou and extremely low in Mengomo (Table 3). In Nkometou, nodule number and mass significantly varied only with variety (Table 2). Though var1, var2 and var5 did not produce significantly large numbers of nodules per plant than var6, yet, nodule mass of var1 was significantly lower than that of var2 (Table 3). Negative, highly significant correlations were established between nodule number and mass ($r= -0.726$; $p< 0.0001$; $n= 68$), between nodule number and FMC ($r= -0.682$; $p< 0.0001$; $n= 68$). However, a positive and highly significant correlation was obtained between FMC and nodule mass ($r= 0.976$; $p< 0.0001$; $n= 68$).

In Mengomo, the Kruskall-Wallis non parametric test showed that nodule number significantly varied with variety and fertilization at 10% level of significance but nodule mass. Though var5 had significantly higher nodule number than the other three varieties, nodule numbers generally were extremely low (Table 3).

Mean grain yield of soybean varieties was about 610 $kg.ha^{-1}$ and 390 $kg.ha^{-1}$ in Nkometou and Mengomo, respectively. Percent variation among sites was on average 42%, 61%, 91% and 57% for var1, var2, var5

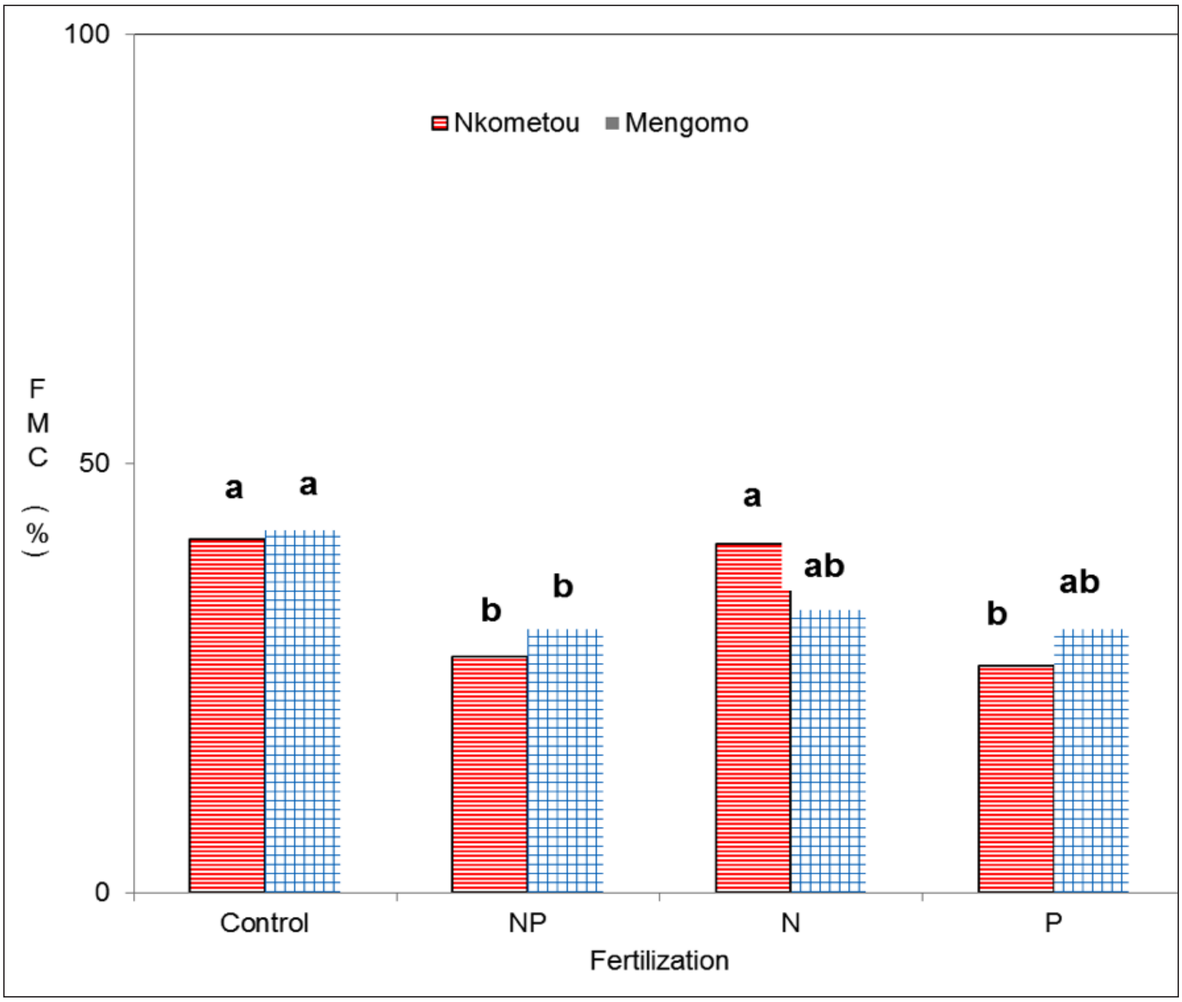

Figure 3: Fractional mycorrhizal colonization (FMC) of soybean roots with N and P fertilization in Nkometou (open bars) and Mengomo (Shaded bars). Bar graphs followed by the same letter are not significantly different at 5% level of significance.

and var6, respectively. Analysis of variation showed generally that grain yield was not significantly affected by all independent variables or by their interactions in both sites but varied highly significantly among fields in Mengomo (Table 2). In Nkometou, correlation between grain yield and nodule mass was positive and significant (r= 0.423; p= 0.001; n= 68).

Plant N content was highly significantly affected by variety while plant P highly significantly varied with fields in Nkometou. Fertilization did not significantly affect plant nutrient uptake (Table 2). Var2 exported the highest plant N content and var6 the least in Nkometou; var1 and var5 were intermediate. Plant N content in Mengomo very highly significantly fluctuated among fields conversely to plant P (p< 0,002**). Correlation between FMC and plant N was negative and very highly significant (r= - 0.682; p< 0.0001; n= 68). Correlation between FMC and plant P was positive, very large and very highly significant (r= 0.976; p< 0.0001; n= 68). Correlation between grain yield and plant P was also positive and highly significant (r= 0.432; p< 0.0008; n= 68).

Discussion

Soil acidity and fertility differed between Nkometou and Mengomo as well symbiotic activities of soybean varieties. Nodulation was significantly affected by soil acidity, conversely to mycorrhizal colonization. In both sites, all soybean root samples reacted to the same extent with the cohorts of indigenous arbuscular mycorrhizal fungi (AMF). Qualitative mycorrhizal colonization did not show neither arbuscules nor hyphal coils but profuse internal hyphae with variously shaped vesicles; auxiliary bodies were also absent on external hyphae. Arbuscules generally indicate functional mycorrhizas but are also ephemeral organs (30), unlikely to be observed in four months old soybean roots. Variously shaped vesicles and diverse abundant internal (both intra- and inter-) hyphae indicate that different glomeromycetous genera of indigenous AMF were involved in the mycorrhizal colonization of soybean roots in these acid, low available P soils.

A functional hypothesis of the various Glomeromycete families has been proposed on the basis of different morphologies and evolutionary histories. It has been suggested that species of the Glomaceae predominantly enhance uptake of immobile elements such as phosphate and those of Gigasporaceae contribute to soil stability (8, 34). The absence of auxiliary bodies but numerous glomacean genera in soybean roots would be consistent with the nodulation requirements for adequate P supply.

In this study, fractional mycorrhizal colonization of soybean roots significantly varied among fields of different fallow ages. In several agroecological zones of Nigeria with comparable soil acidity levels, promiscuous soybean varieties showed similar levels of mycorrhizal colonization (about 20 to 40%) in fields with different cropping histories (26, 28). In tropical soils of West and Central Africa, promiscuous soybean varieties were found moderately mycorrhizal (20). Yet, in acid soils of Cameroon, soybean varieties benefited from native mycorrhizal symbionts for P supply much required in the nodulation process by native strains of *Bradyrhizobia*. In Nkometou, a highly significant positive correlation was obtained between mycorrhizal colonization and nodule mass.

Fractional mycorrhizal colonization significantly increased in fields with increasing fallow ageing and in forest regrowth, implying a buildup in arbuscular mycorrhizal inoculum potential during vegetative fallowing, as observed earlier (24). Fallowing with *Chromoloana odorata* may boost AM inoculum which in return could improve the availability of slowly mobile nutrients such as phosphate (23) or fast moving ones like potassium (18, 31). Local shifting cultivators claimed that productivity of local peanut (*Arachis hypogea*) varieties always increased after vegetative fallowing with *C. odorata*. However, mycorrhizal activities of this exotic “weed” species in enhancing nutrients’ availability for subsequent crops largely remain unknown in the maintenance of soil fertility in the tropics.

After P fertilization, fractional mycorrhizal colonization of soybean roots was reduced in soils of both sites, to different extent, though. Earlier findings showed that in soils with very high or very low P availability, mycorrhizal infectivity was depressed (19). Though soil P availability after fertilization was not assessed, it is likely that soil solution P differed in the two sites with regard to their soil reaction and levels of calcium (Table 1).

Nitrogen applications resulted in contrasting effect on fractional mycorrhizal colonization: increased in the near neutral soils of Nkometou and decreased in the strongly acid soils of Mengomo, along the lines with earlier reports (5). But the direction of N effect has been observed to depend on plant P nutrition (32) while the intensity effect was influenced by the balance of N and P (17), the source of N and the acidifying effect of N on the rhizosphere pH (21). In this study, fertilization did not significantly affect plant P in both soils. The contrasting observations from both sites could originate from urea’s double acidifying effect, exacerbated in the acid soils of Mengomo.

Nodulation significantly varied with site. High nodulation in Nkometou strongly contrasted with extremely low nodule numbers in Mengomo, implying the existence of suitable native strains of acid-tolerant *Bradyrhizobia* in some Cameroonian acid soils. Therefore, some soybean varieties can be successfully introduced in parts of the humid forest margins of Cameroon without need for bradyrhizobial inoculation and initial fertilization.

Nodulation and nodule activities require sufficient phosphate nutrition (14). Because AM fungi can satisfy this condition under otherwise limiting P regimes, the positive effects of AM fungi on biological N_2 fixation have been traditionally ascribed to increased P to nodules (15). Mycorrhizal soybean had increased N_2 fixation with enhanced number and dry weight of nodules, N content and N_2 fixed (33). In Nkometou, nodule mass was strongly and positively correlated with mycorrhizal colonization. The negative correlation with nodule number could be due to empty nodules.

Nodulation was not significantly affected by fertilization but only by variety in Nkometou. Though var1, var2 and var5 produced equally the same number of nodules, they significantly differ in nodule mass. Var2 and var5, early maturing varieties, produced the highest nodule mass while var6 yielded the lowest nodule number and mass, and exported the least N content. Accordingly, var6 should not be recommended for release to farmers in Nkometou. Var1 which is a late maturing variety was not as equally productive as early maturing varieties, mainly with regard to N uptake. Among the two early maturing soybean varieties, var2 may be recommended for the Nkometou area if subsequent bioassays show that nodulation of var2 is the most actively N_2-fixing. Definitely, var2 should not be recommended for acid soils such as those prevailing in Mengomo.

Similar fertilizer applications resulted in various grain yields in both sites. Grain yield was higher in Nkometou than in Mengomo. Agricultural productivity in acid soils is often lower compared with well-buffered soils. Difference in biological nitrogen fixation could explain drop in grain yield in Mengomo since mycorhization was not dissimilar in both sites. There was no response to fertilizer applications in grain yield of nodulating and non nodulating soybean varieties. Our results support the view that there was no justification for applying starter fertilizer N or P or both at this rate to these acid humid soils of Cameroon to grow these soybean varieties owing to the existence effective native strains of *Bradyrhizobium* and arbuscular mycorrhizal fungi. Such conclusions had been reached by earlier workers elsewhere (1). In Nkometou, grain yield was not significantly affected by independent variables and their interactions. However, correlation between grain yield and symbiotic parameters indicated that, at least, 40% of grain yield in Nkometou could be accounted for biological nitrogen fixation (BNF) which in turn was positively and highly significantly correlated with mycorrhizal colonization by indigenous AM fungi. There was also a very highly significant correlation between FMC and plant P content. In Mengomo, a highly positive correlation was obtained between FMC and grain yield which depicts the significance of symbiotic activities, in particular, those of indigenous mycorrhizal fungi, for acid soil productivity. In Brazil, BNF accounted for to 40% for grain yield of soybean (4).

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